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Title: The Influence of Surface Lubricity on the Adhesion of *Navicula perminuta* and *Ulva linza* to Alkanethiol Self-Assembled Monolayers

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Abstract: The settlement and adhesion of *Navicula perminuta* and *Ulva linza* to methyl-terminated alkanethiol self-assembled monolayers (SAMs) of increasing chain length has been investigated. Organisms were allowed to settle onto the monolayers and were subsequently exposed to hydrodynamic shear stress in order to determine their adhesion strength. Results show that as the SAM structure changes from amorphous to crystalline (C14) there is a marked change in the adhesion of *Navicula perminuta* and *Ulva linza*. Given that the SAMs in the series all exhibit similar contact angle behaviour and surface energy, it is hypothesised that the lubricity of the surface plays a role in determining the surface adhesion.

Keywords: adhesion, biofouling, alkanethiol, self-assembled monolayer, *Ulva*, *Navicula*, lubricity.

## 1. INTRODUCTION

Marine biofouling, the colonisation of submerged structures by barnacles, macroalgae and microbial slimes, has major economic implications for military and commercial shipping (Callow and Callow 2002). Finding environmentally benign methods of controlling biofouling requires a greater understanding of the settlement, colonisation and adhesion processes used by fouling organisms to select and adhere to submerged surfaces. For such purposes the advantages of using well-defined model surfaces based on self-assembled monolayers has been well-documented (e.g. Callow et al., 2000, 2005; Ista et al., 2004).

*Ulva linza*, a major macroalgal fouling species in temperate zones, colonises new surfaces by the release of quadriflagellate zoospores. Spores swim freely in the water column, and on contacting a suitable surface secrete a pre-formed, fast curing glycoprotein adhesive that surrounds the spore anchoring it to the surface (Callow *et al.* 1997). Once the adhesive is released the spore is permanently secured to the substratum. Diatoms, a family of siliceous microalgae, form a major component of marine microbial biofilms. The method used by diatoms to colonise a new surface is quite distinct from that of the macroalgae. When in suspension diatoms are non-motile, lacking flagella to swim with. They colonise new surfaces by passive processes such as gravitational settlement and adhere through the production of a polysaccharide based extracellular polymeric substance (EPS) (reviewed by Chiovitti et al. 2006). Once adhered to a surface diatoms are capable of a ‘gliding’ motility mediated by the production of EPS from a slit in the cell termed the raphe.

The physical properties of a surface have been shown to have a profound effect on the settlement and adhesion of fouling organisms. Microtopography and surface roughness influence both the settlement (Callow *et al.* 2002) and attachment strength (Granhag *et al.* 2004) of *Ulva linza* spores. Similarly surface energy and wettability have been shown to influence the settlement behaviour of *Ulva* zoospores (Callow *et al.* 2000), the attachment of marine bacteria *Cobetia marina* (Ista *et al.* 2004) and the attachment strength of *Ulva linza* and the diatom *Amphora* spp. (Finlay *et al.* 2002). It is proposed that the influence of wettability is derived from differences in adhesive spreading on the surface altering the contact area (Callow *et al.* 2005). The influence of surface lubricity and modulus has also been shown to be of significance in determining the attachment strength of *Ulva* spores (Chaudhury *et al.* 2005; Hoipkemeier-Wilson *et al.* 2004).

In this paper, the settlement and adhesion of spores of *Ulva linza*, and a diatom *Navicula perminuta*, to methyl-terminated alkanethiol SAMs of different chain length has been investigated. Compounds **1-6** (**Figure 1**) are alkanethiols varying in length from an octyl chain to an octadecyl chain, each compound being increased in length by one ethylene unit from the previous adsorbate. SAMs formed from compounds **1-6** all present a methyl-terminated surface to the aqueous phase. SAMs formed from compounds **1-6** exhibit advancing water contact angles in the region 111-115 $^{o}$. It is known that SAMs formed from compounds **1** ($C_8H_{17}SH$) and **2** ($C_{10}H_{21}SH$) exhibit a significant degree of alkyl chain mobility, whereas SAMs formed from compounds **3** ($C_{12}H_{25}SH$) to **6** ($C_{18}H_{37}SH$) are two-dimensional quasi-crystalline solids (Porter *et al*. 1987; Evans *et al*. 1991; Evans & Ulman 1990). As a result, SAMs formed from compounds **1** and **2** have alkyl chain structures that possess higher densities of *gauche* defects and are more readily deformable than those of **3-6**. Thus, the objective of investigating this SAM series was to assess how settlement and adhesion of *Ulva linza* and *Navicula perminuta* is affected by changes in SAM structure as the chain length is increased.

## 2. MATERIALS AND METHODS

### *2.1 SAMs*

Au substrates were prepared by thermally evaporating (Edwards Auto 306 Evaporator) a Cr adhesion promoter (~ 5nm) onto clean glass slides (BDH) followed by ~ 100 nm of Au Deposition was monitored using a quartz crystal microbalance (QCM) thickness monitor employing deposition rates in the range 0.05-0.10 nm $s^{-1}$ for both Au and Cr. All glassware used in SAM formation was cleaned prior to use by immersion in 'piranha' solution, a 3:7 mixture of 30% hydrogen peroxide (Fisher Scientific, laboratory reagent grade) and concentrated sulfuric acid (Fisher Scientific, analytical reagent grade), at room temperature for ~ 1 h. Cleaning with piranha solution was followed by rinsing with copious amounts of 18 MΩ deionised $H_2O$ (Elga UHQ-PS) and drying in an oven at 140 $^{o}$C. All Au substrates were cleaned prior to SAM formation by immersion in piranha solution at room temperature for 10 minutes. Cleaning with piranha solution was followed by rinsing with copious amounts of 18 MΩ deionised $H_2O$ (Elga UHQ-PS) and rinsing with copious amounts of $C_2H_5OH$. SAMs were prepared by immersing Cr-primed, Au-coated glass microscope slides in 1 mM $C_2H_5OH$ (Fisher Scientific, HPLC grade) solutions of the SAM compounds for 24 h. The Au substrates were removed from the SAM solution and rinsed with copious amounts of $C_2H_5OH$, before being blown dry using Ar gas.

Dynamic $H_2O$ contact angles were measured using a custom-made stage apparatus, employing a Charge-Coupled Device (CCD) KP-M1E/K camera (Hitachi) and FTA Video Analysis software v1.96 (First Ten Angstroms) for analysis of the contact angle of a droplet of UHQ $H_2O$ at the three-phase intersection point. All data was collected under conditions of ambient temperature, pressure and humidity. A minimum of 7 measurements were performed for each sample.

Ellipsometry measurements were performed using a multi-spectroscopic ellipsometer (Jobin-Yvon/Horiba) operating with DeltaPsi2 v2.0.8 software. The angle of incidence was set to 70 $^{o}$. The light wavelength range used for all measurements was 280-800 nm. All measurements were made under conditions of ambient temperature, pressure and humidity. SAM thicknesses are averages of a minimum of six measurements, each made at a different location on the substrate.

XPS analysis of SAMs was performed using an Escalab 250 system (Thermo VG Scientific) operating with Avantage v1.85 software. An Al Kα X-ray source was used, providing a monochromatic X-ray beam with incident energy of 1486.68 eV. All measurements were made at a pressure of $< 5 \times 10^{-9}$ mbar. Detailed scans of Au $4f_7/4f_5$ (86 eV), S 2p (163 eV) and C 1s (286 eV) were performed using a pass energy of 20 eV and a step size of 0.1 eV.

### *2.2 Settlement and removal of* Ulva linza *and* Navicula perminuta

Six replicate SAMs of each chain length were used for the *Ulva* and the *Navicula* assay. All SAMs were rinsed in artificial seawater at pH 8.2 immediately prior to use. Reproductive plants of the green macroalga *Ulva linza* were collected from Wembury beach, Devon, England. Zoospores were released and the assay conducted according to the protocols detailed in Callow *et al.* (1997). Zoospores suspensions containing 1.5 x $10^6$ spores $ml^{-1}$ were allowed to settle onto SAMs for 45 min in the dark. Slides were rinsed to remove unsettled zoospores and three replicates were then fixed and washed as described in Callow *et al.* (1997). After rinsing, the remaining three replicates were exposed to 54 Pa wall shear stress in a fully turbulent water-channel (Schultz *et al.*, 2000) before fixation and washing. Spore density on the SAMs before and after exposure to flow was determined as described in Callow *et al.* (2002), utilising the autofluorescence of chlorophyll to locate settled spores with a Zeiss Axioskop 2 fluorescence microscope.

Cultures of the pennate diatom *Navicula perminuta* (Bacillariophyceae), originally isolated by Dr R Wetherbee, were grown in F2 medium at 18°C on a 16 h: 8 h light: dark cycle. Exponentially growing cultures of *Navicula* were prepared and assayed as detailed in Pettitt *et al.* (2004). A cell suspension of 0.32µg chla $mL^{-1}$ was allowed to settle onto SAMs for 120 min, before slides were rinsed and processed as for the *Ulva* assay above. Cell density before and after flow was determined by manual counts using a Olympus BH2 transmitted light microscope.

## 3. RESULTS

SAMs formed from compounds **1-6**, which are simple n-alkanethiols, will exhibit similar contact angles similar surface energies (Bain *et al*. 1989). Leggett (2003) reported differences in the frictional properties of alkanethiol SAMs of increasing chain length established using atomic force microscopy. The frictional properties of alkanethiol SAMs may be quantified by determining their coefficients of friction (μ), defined as the gradient of a graph of the friction force plotted as a function of the load applied perpendicular to the sample (Leggett *et al*. 2005). Friction coefficients for the SAMs studied here are shown in Table 1. The largest coefficient of friction was measured for the shortest adsorbate, and the value of μ decreases as the alkyl chain length increases, in agreement with previous studies (McDermott *et al*. 1997).

The density of *Ulva* spores and *Navicula* cells prior to and post exposure to 54 Pa wall shear stress is shown in **Figures 2** and **3** respectively. The density of *Ulva* spores initially settled on the different SAMs was very similar but there appears to be a minor trend of increasing initial attachment of *Navicula* cells with reducing alkanethiol chain length. Irrespective of the level of initial settlement, when the adhesion strength of the attached cells of both organisms was examined by measuring the proportion of attached cells removed under flow, as a function of SAM friction coefficient, a clear trend was observed for both organisms (**Figure 4**). Removal was approximately constant for friction coefficients greater than 0.3 (SAMs formed from compounds **1-3**), but increased with decreasing friction coefficient for friction coefficients less than 0.3.

## 4. DISCUSSION AND CONCLUSIONS

It has previously been established that long alkyl chain moieties within a SAM promote well-ordered molecular packing, while shorter chain lengths promote a loss of molecular organisation (Porter *et al*. 1987; Evans *et al*. 1991). Evans and Ulman (Evans & Ulman 1990) stated that SAMs formed from alkanethiols with chain lengths less than 10 methylene units would be disordered, while SAMs formed from alkanethiols with chain lengths greater than 12 methylene units would be ordered. Hence, SAMs formed from the shorter chain compounds, **1** and **2**, exhibit higher densities of *gauche* defects and more mobile alkyl chains than those SAMs formed from compounds **3-6**. At a chain length of 12 methylene units, the monolayer is thought to adopt a two-dimensional crystalline arrangement in which the adsorbates are almost entirely *trans*-extended. This point corresponds to the onset of increasing organism removal in **Figure 4**. It is proposed that this change in surface structure is the cause of the increase in organism removal.

The adhesion of *Navicula perminuta* and *Ulva linza* to a surface relies on the secretion of adhesive molecules which surround the cell and wet the surface. The chemical and physical properties of a surface will affect the interaction of this adhesive with the surface. Lee *et al*. (Lee *et al*. 2000) reported that shorter, less well-ordered SAMs exhibit greater frictional forces, as measured using AFM, than crystalline, ordered SAMs, discussing the effect of intermolecular packing on the modulus of the SAM. A disordered SAM will have a lower modulus than a crystalline SAM and therefore can provide a greater surface area for interaction, in this case with the adhesive of a *Navicula* cell or

*Ulva* spore. On the application of a shear stress, we may therefore anticipate lower removal from the amorphous SAM as a result of the increased area over which the diatom or spore is adhered.

Additionally, the reaction of the SAM surface to the application of energy (shear stress) may also affect the removal of the adhered spores / cells. The less well ordered, short-chain SAMs will provide a greater number of channels for energy dissipation than crystalline SAMs, for example through molecular motions such as the formation of gauche defects and rotation of carbon-carbon bonds. Due to the increased number of van der Waals forces between chains in crystalline SAMs, which will increase with increasing chain length, greater energy input is required to deform these SAMs. When an attached diatom or spore is subjected to a shear stress, if the surface onto which it has adhered is amorphous, more energy will be dissipated through the surface than if the surface were crystalline. Given that the EPS secreted by a cell has a constant strength on either a crystalline SAM or an amorphous SAM, it appears that cells on amorphous SAMs will adhere more strongly than cells on crystalline SAMs. This may explain why reduced adhesion strength (i.e. higher removal under shear stress) of *Navicula* and *Ulva* is seen from the crystalline SAMs than from the amorphous SAMs.

In conclusion, the adhesion of *Navicula perminuta* and *Ulva linza* to alkanethiol SAMs was found to decrease with increasing alkyl chain length for SAMs with alkyl chain lengths greater than 12 methylene units, which are two-dimensional quasi-crystalline solids. It is proposed that as the alkyl chain length increases the dissipation of energy through the SAM, upon application of a hydrodynamic force, becomes less favourable, leading to greater disruption of the adhesive bond between the EPS secreted by the organism and the SAM. Hence, the adhesive bond fails more readily for longer alkyl chain lengths.

## 5. ACKNOWLEDGEMENTS

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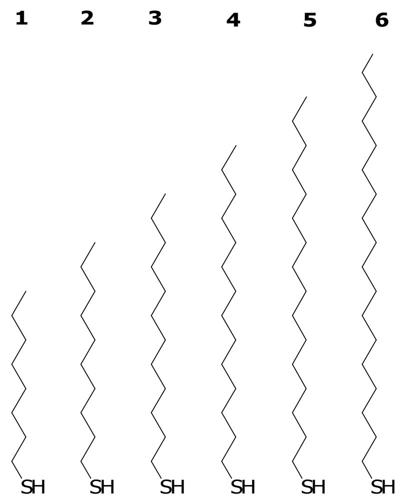


**Figure 1:** Schematic of the molecular structures of compounds **1-6**

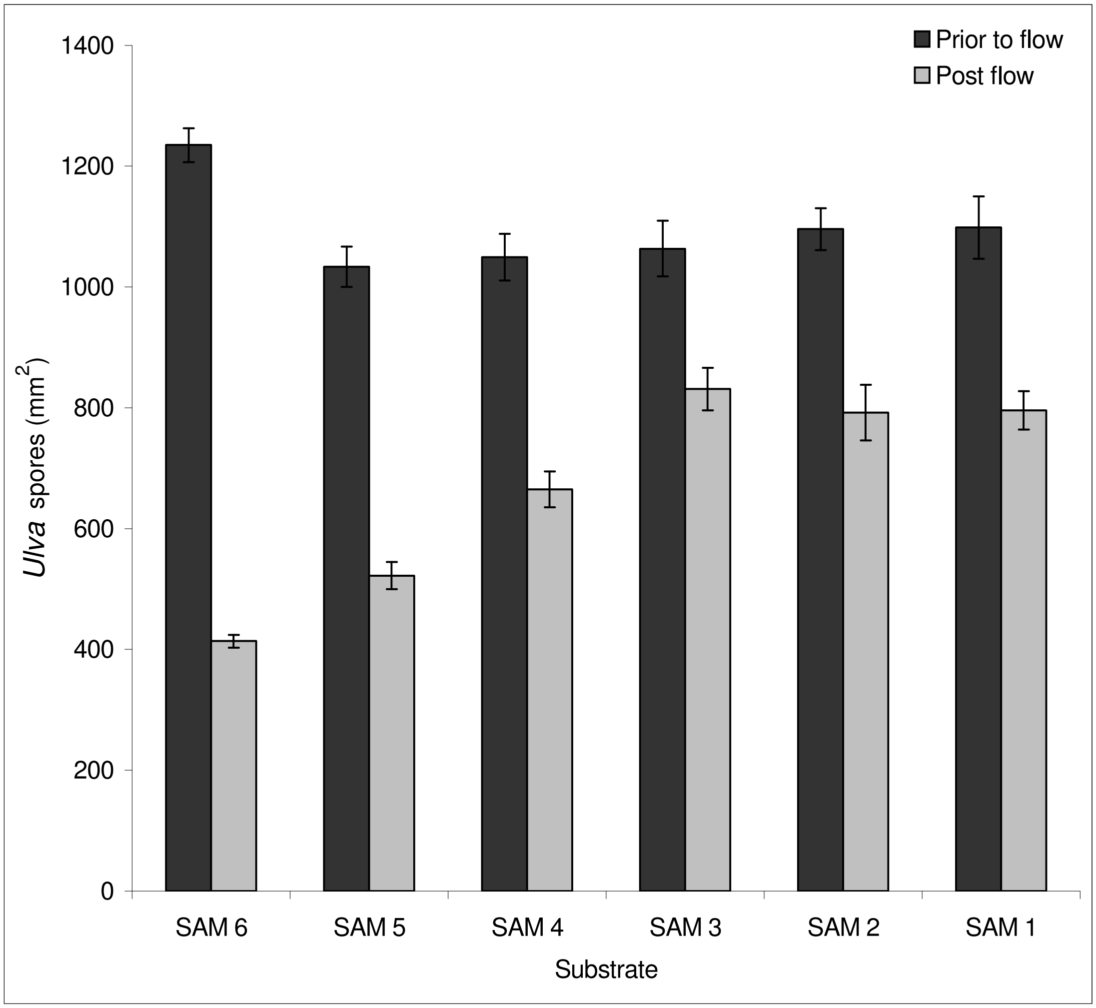


**Figure 2:** Density of attached *Ulva* spores on SAMs of varying chain length, prior to (dark columns) and post (light columns) exposure to 54 Pa wall shear stress. N = 30, error bars = ± 2 x Standard Error.

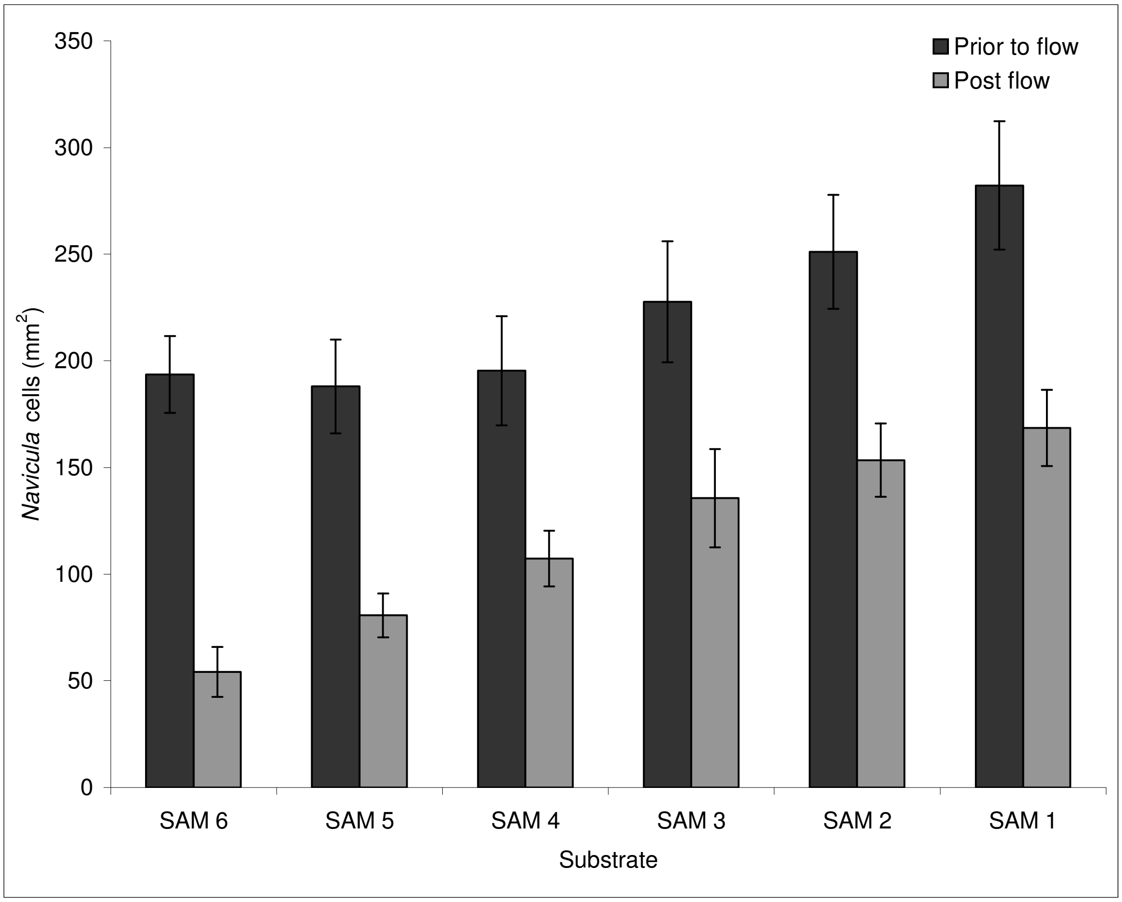


**Figure 3:** Density of attached *Navicula* cells on SAMs of varying chain length, prior to (dark columns) and post (light columns) exposure to 54 Pa wall shear stress. N = 30, error bars = ± 2 x Standard Error.

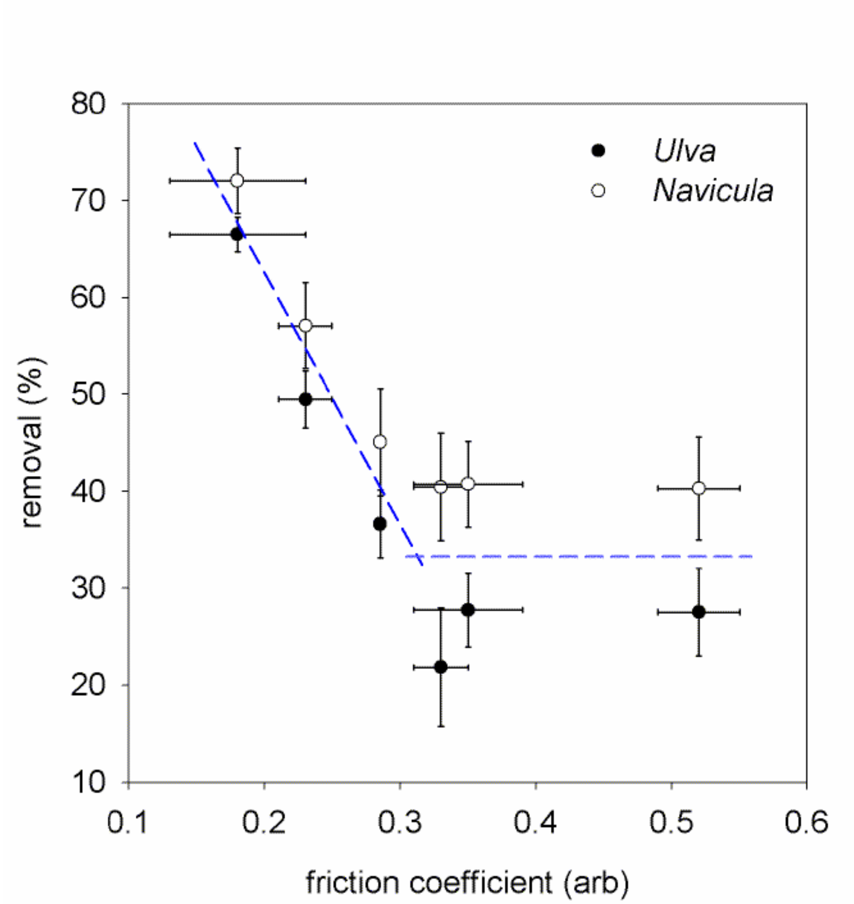


**Figure 4:** Removal (%) of *Navicula* cells and *Ulva* spores by 54 Pa wall shear stress, as a function of SAM friction coefficient. Removal: N = 30, error bars = 2 x Standard Error derived from arcsine transformed data. As the friction coefficient of compound **4** was not measured by Leggett (2003) a value has been interpolated from the equation $y = -0.0301x + 0.7067$ ($r^2 = 0.9051$).

| Alkyl chain length | Advancing contact angle (°) | Friction coefficient |
|---|---|---|
| 8 | 111 ± 1 | 0.52 ± 0.03 |
| 10 | 113 ± 1 | 0.35 ± 0.04 |
| 12 | 113 ± 1 | 0.33 ± 0.02 |
| 16 | 115 ± 1 | 0.23 ± 0.02 |
| 18 | 115 ± 1 | 0.18 ± 0.05 |

**Table 1:** Measured advancing water contact angles and friction coefficients for SAMs formed from compounds **1**, **2**, **3**, **5** and **6 (Leggett, 2003)**

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